An Adjuvanted Polyprotein HIV-1 Vaccine Induces Polyfunctional Cross-Reactive CD4+ T Cell Responses in Seronegative Volunteers

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Background. This phase I/II partially blinded, randomized, dose-ranging study assessed the safety and immunogenicity of a novel human immunodeficiency virus type 1 (HIV-1) vaccine candidate consisting of a recombinant fusion protein (F4) containing 4 HIV-1 clade B antigens (Gag p24, Pol reverse transcriptase, Nef, and Gag p17) adjuvanted with AS01 in HIV-seronegative volunteers.

Methods. Two doses of the recombinant F4 protein (10, 30, or 90 μg/dose), adjuvanted with AS01 or reconstituted with water for injection, were administered 1 month apart to 180 healthy volunteers aged 18–40 years. F4-specific $CD4^+$ T cell responses were measured using intracellular cytokine staining after in vitro stimulation by overlapping peptide pools covering the 4 individual antigens.

Results. Reactogenicity was higher during the 7-day period after each vaccine dose in the adjuvanted than in the nonadjuvanted groups. In the adjuvanted groups, the overall immune response rate was high after the second vaccine dose, with highest responder rates seen in the 10-μg F4/AS01 group (100% to 3 HIV-1 antigens and 80% to all 4 HIV-1 antigens). High and long-lasting $CD4^+$ T cell frequencies were observed (up to a median value of 1.2% F4-specific $CD4^+$ T cells at day 44), with strongest responses directed against reverse transcriptase. Antigen-specific $CD4^+$ T cells exhibited a polyfunctional phenotype, expressing at least CD40 ligand and interleukin 2, often in combination with tumor necrosis factor α and/or interferon γ. Vaccine-induced $CD4^+$ T cell responses were broadly cross-reactive to all 4 antigens derived from HIV-1 clades A and C.

Conclusions. These results support further clinical investigation of this HIV-1 vaccine candidate both in a prophylactic setting (alone, in conjunction with an envelope-based antigen or in combination with other vaccine approaches in a heterologous prime-boost regimen) and as a potentially disease-modifying therapeutic vaccine in HIV-1–infected subjects.

Clinical trials registration. NCT00434512.

Development of a safe and effective prophylactic vaccine against human immunodeficiency virus type 1 (HIV-1) is a global health priority [1]. Three candidate HIV-1 vaccines have been tested in phase IIb or III trials. An adenovirus vector-based vaccine and bivalent recombinant gp120 protein vaccines failed to prevent HIV-1 infection [2–4], but a combination of a poxvirus vector and recombinant gp120 proteins recently demonstrated modest protection [5]. Although the most desirable goal of an HIV-1 vaccine remains the prevention of infection, a disease-modifying vaccine inducing strong T cell–mediated immune responses remains a valuable alternative.

The role of $CD8^+$ T cell responses in controlling persistent virus infections is well established [6–9]. Virus-specific $CD4^+$ T cells also play a central role in the immune control of many viral infections, including HIV-1 [10]. $CD4^+$ T cells are required for the induction

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and maintenance of functional $CD8^+$ T cells [11–14], and the presence of polyfunctional and proliferation-competent HIV-1–specific $CD4^+$ T cells in HIV-1–infected patients is associated with long-term nonprogression (LTNP) [15–17]. Loss of HIV-1–specific $CD8^+$ T cell proliferation after acute HIV-1 infection can be restored by vaccine-induced HIV-1–specific interleukin (IL) 2–producing $CD4^+$ T cells in vitro and in vivo [18].

A previous HIV-1 vaccine candidate comprising gp120 and a NefTat fusion protein formulated in proprietary immunostimulatory Adjuvant Systems elicited strong $CD4^+$ T cell responses in healthy HIV-seronegative adults [19, 20] and in HIV-1–infected subjects receiving antiretroviral therapy [21]. Based on these findings and promising results with malaria [22–24] and recombinant hepatitis B virus [25] antigens, AS01—a liposome-based Adjuvant System containing 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) and QS21—was chosen for further investigation due to its propensity to induce a stronger $CD4^+$ T cell response [20, 25, 26].

Because vaccine-induced $CD4^+$ T cell responses need to cover the broadest possible spectrum of circulating HIV-1 strains, an HIV-1 vaccine should contain as many $CD4^+$ T cell epitopes as possible from different viral proteins [27]. The viral antigens containing the highest number of conserved T cell epitopes are Gag, Pol, and Nef [28]. Given their role in HIV-1 pathogenesis and as targets for $CD8^+$ T cell responses [29, 30], p17 and p24 encoded by *gag*, reverse transcriptase (RT) encoded by *pol*, and the regulatory protein Nef have been included as a single fusion protein (F4) in a novel vaccine formulation. This study evaluated the safety and immunogenicity of the F4 protein antigen adjuvanted with AS01 in healthy HIV-seronegative volunteers.

MATERIALS AND METHODS

Study Vaccine

The HIV-1 vaccine candidate contained 10, 30, or 90 μg of F4 recombinant protein per dose as an active ingredient, adjuvanted with AS01 or reconstituted with water for injection (WFI). F4 is a recombinant fusion protein expressed in *Escherichia coli* and comprising 4 HIV-1 clade B antigens: p24 (BH10), RT (HXB2), Nef (Bru-Lai), and p17 (BH10). The vaccine antigen was prepared as a lyophilized pellet containing F4 in sucrose, ethylenediaminetetraacetic acid, arginine, polysorbate 80, and sodium sulfite in phosphate buffer. AS01 is a liposome-based Adjuvant System containing 50 μg of MPL and 50 μg of QS21. The freeze-dried fraction containing the F4 antigen and the liquid fraction consisting of AS01 or WFI, both presented in a single-dose 3-mL glass vial, were reconstituted by an unblinded vaccinator, and .5 mL of the reconstituted vaccine solution was injected into the deltoid muscle of the subject's nondominant arm.

Study Design and Participants

This was a phase I/II, single-center, partially blinded, parallel-group study with a dose-escalating, staggered design (NCT00434512). The study was approved by the local independent ethics committee and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and all subjects provided written informed consent.

Participants were healthy male and female adults, aged 18–40 years; at low risk of HIV infection; seronegative for antibodies against hepatitis B core antigen, hepatitis C virus, HIV-1 and HIV-2 and negative for hepatitis B surface antigen and HIV p24 antigen in screening serum samples obtained 8 weeks before vaccination (all tests from Abbott AxSYM). Standard eligibility criteria were used for enrollment, as detailed in the ClinicalTrials.gov registry.

Subjects were randomized (5:1) to receive either AS01 ($n = 50$) or WFI ($n = 10$) for each F4 dose (10, 30, or 90 μg). The observers were blinded for adjuvantation but not for antigen content. Each subject received 2 vaccine doses 1 month apart. Blood samples were obtained before vaccination (day 0), 2 weeks (day 44) and 1 month (day 60) after the second vaccine dose, and at months 6 (day 180) and 12 (day 360). All laboratory assays were performed with blinding.

Safety

Solicited local (injection site pain, redness, swelling) and general symptoms (fever, fatigue, headache, sweating, myalgia, gastrointestinal symptoms) were recorded on diary cards for 7 days after each vaccination. Symptom severity was graded on a scale of 1–3, with grade 3 symptoms defined as redness or swelling >50 mm, fever >39.0°C, and any other symptom preventing normal daily activities. Unsolicited symptoms were recorded for 30 days after each vaccination. Serious adverse events were recorded throughout the study.

T Cell Responses

T cell responses were evaluated by intracellular cytokine staining after in vitro stimulation with p17, p24, RT, and Nef peptide pools to assess the expression of IL-2, interferon (IFN) γ, tumor necrosis factor (TNF) α, and CD40-ligand (CD40L) with an adaptation of a previously described method [31], using peripheral blood mononuclear cells (PBMCs) isolated from venous blood. In brief, thawed PBMCs were stimulated in vitro with pools of 15-mer peptides overlapping by 11 amino acids (Eurogentec) covering the sequences of clade B p17, p24, RT, or Nef matched antigens or medium only in the presence of anti-CD28 and anti-CD49d antibodies. After 2 hours at 37°C, brefeldin A was added to inhibit signal molecule secretion during an additional overnight incubation. Cells were harvested, stained for surface markers (CD4 and CD8), fixed, permeabilized, and stained with labeled antibodies to IL-2, IFN-γ, TNF-α, and CD40L (all reagents, BD Biosciences). Flow cytometric analysis

was performed with a FACSCanto flow cytometer and FACS-Diva Version 6.1.1 (BD Biosciences) or FlowJo Version 8.8.2 (Tree Star) software.

To assess cross-reactivity of vaccine-induced $CD4^+$ T cells with non–clade B HIV-1 antigens, PBMCs collected at days 0 and 44 were analyzed for expression of CD40L and intracellular production of IL-2, IFN-γ, and TNF-α, using peptide pools from clade A (p17 and p24 from TZA173 [Tanzania]; RT and Nef from KE MSA4070 [Kenya]) and C (ZM651) HIV-1 strains. Clade B antigens were included in the same assay as controls. This exploratory analysis was performed only on samples from subjects in the 10-μg F4/AS01 group. Amino acid alignment analysis was performed using Clustal W2 (Lasergene) and Jalview Version 2 [32] software. A subject was considered a responder if ⩾.03% antigen-specific $CD4^+$ T cells were observed after background subtraction. This cutoff was selected based on the maximum value of all 95th percentiles for the percentage of antigen-specific $CD4^+$ T cells expressing ⩾2 markers before vaccination.

Humoral Immune Response

Immunoglobulin G (IgG) antibody responses to F4, p17, p24, RT, and Nef were analyzed by using standard in-house enzyme-linked immunosorbent assays. Antibody concentrations were calculated by comparing the dose-range curve from the analyzed sample with that of an in-house reference sample. All assays included negative and positive internal controls. The cutoff for seropositivity was ⩾187 mEU/mL for p17, ⩾119 mEU/mL for p24, ⩾125 mEU/mL for RT, ⩾232 mEU/mL for Nef, and ⩾42 mEU/mL for F4.

Statistical Analysis

Analysis of safety was performed on the total vaccinated cohort. The number and percentage of subjects reporting solicited and/or unsolicited local and general symptoms were calculated with exact 95% confidence intervals (CIs).

Analysis of immunogenicity was performed on according-to-protocol cohorts at months 2 and 12. The frequency of $CD4^+$ T cells expressing IL-2 and ⩾1 other marker and the percentage of responders after in vitro stimulation to each individual antigen and to at least 1, 2, 3, and all 4 antigens were determined at each time point. The F4-specific $CD4^+$ T cell response was estimated from the sum of the specific $CD4^+$ T cell frequencies in response to each individual antigen. In the adjuvanted groups, F4-specific $CD4^+$ T cell frequencies 2 weeks after the second vaccination were compared between doses by 1-way analysis of variance using the $\log_{10}$ frequencies, with dose (10, 30, and 90 μg) included as a fixed effect, followed by a Tukey adjustment. Statistical comparisons between groups were not performed at any other time.

Seropositivity rates and geometric mean antibody concentrations (GMCs) for F4 and each individual antigen were calculated with 95% CIs, using the exact method for binomial variables for seropositivity rates and the antilogs of the 95% CIs

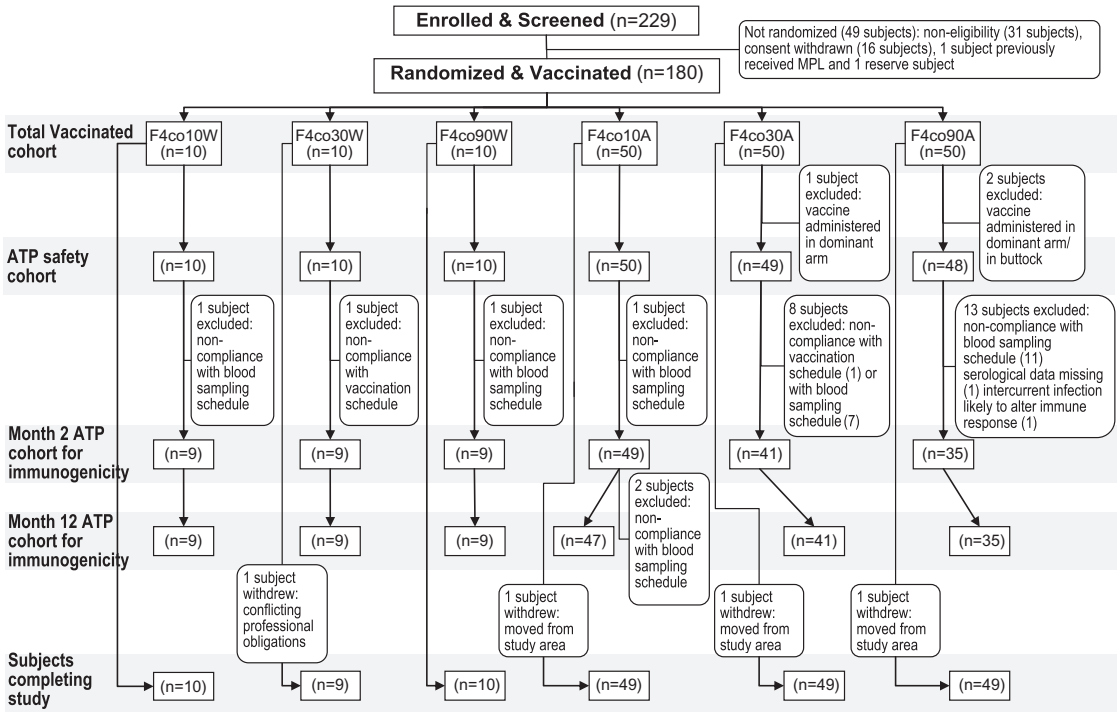

Figure 1. CONSORT flow diagram. ATP, according to protocol.

of the mean $\log_{10}$-transformed antibody concentrations for GMCs. Antibody concentrations below the assay cutoff were given an arbitrary value of half the cutoff for the GMC calculation.

RESULTS

Demographics

The mean (standard deviation) age of study participants was 22.3 (4.62) years, 63.3% were female, and 96.7% were white. No differences in baseline demographics were observed between groups. All 180 subjects received both vaccine doses, and 176 completed the study. The according-to-protocol cohort for analysis of immunogenicity comprised 152 subjects (84.4%) at month 2 and 150 (83.3%) at month 12 (Figure 1).

Safety

Reactogenicity was higher during the 7-day period after each vaccine dose in the F4/AS01 groups than in the F4/WFI groups. The incidence of local and general symptoms tended to be higher in the F4/AS01 groups after the second vaccine dose (Figure 2). Pain was the most common solicited local symptom, reported after 94.0%–98.0% of doses in the F4/AS01 groups and after 0%–40.0% of doses in the F4/WFI groups (grade 3 severity after 2.0%–12.2% of doses in the F4/AS01 groups). Fatigue was the most common solicited general symptom, reported after

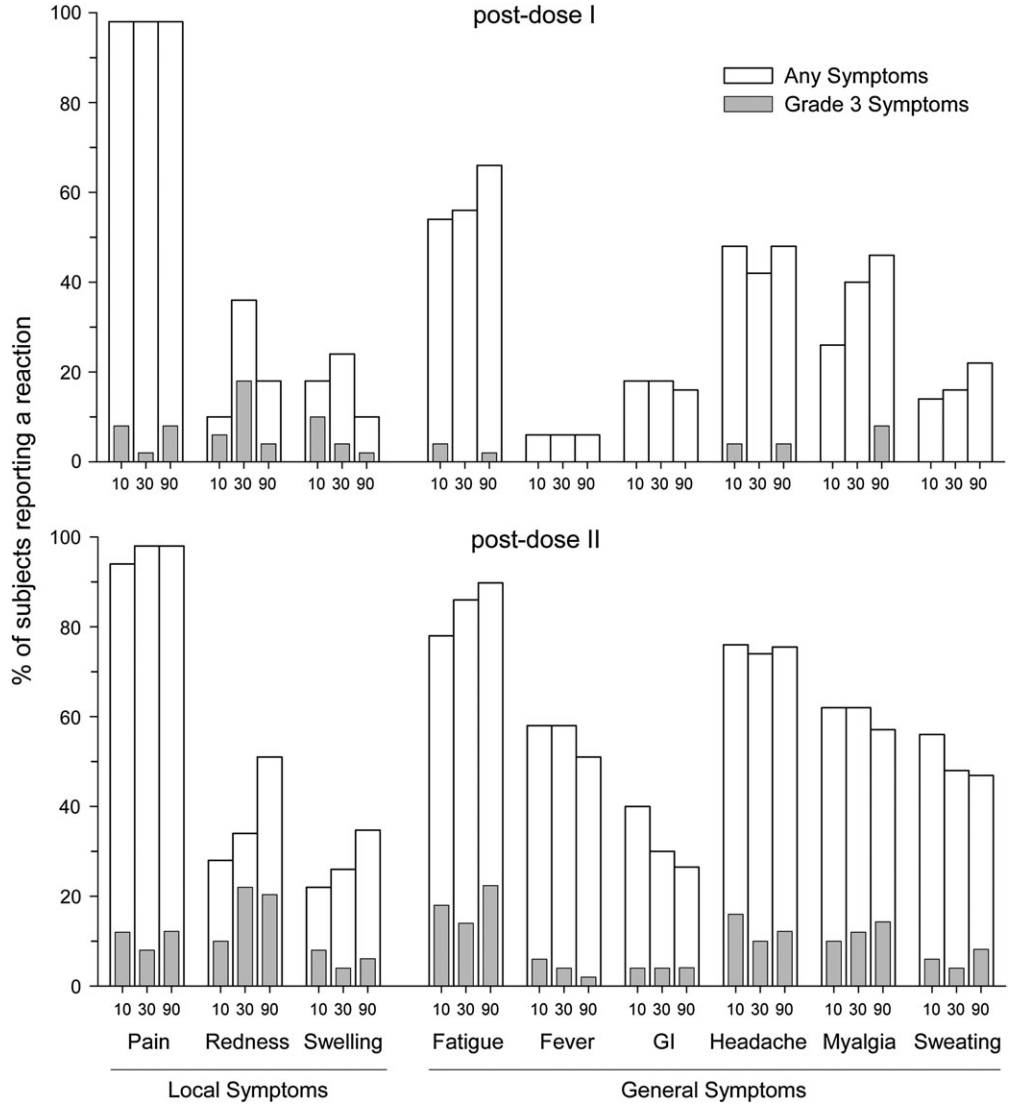

Figure 2. Incidence of solicited local and general symptoms (any and grade 3 severity) during the 7-day period after each vaccine dose. Solicited local symptoms (injection site pain, redness, swelling) and general symptoms (fatigue, fever, gastrointestinal [eg, nausea, vomiting, diarrhea, and abdominal pain], headache, myalgia and sweating) symptoms were recorded on diary cards for 7 days after each vaccine dose. Symptom severity was graded on a scale of 1–3, with grade 3 symptoms defined as redness or swelling >50 mm in diameter, fever >39.0°C, and any other symptom preventing normal daily activities.

Table 1. $CD4^+$ T Cell Response to the F4/AS01-Adjuvanted Human Immunodeficiency Virus Type 1 Vaccine Candidate: Responder Rates[a]

Time Point	F4 Dose, μg	Subjects, No.[b]	Responders by No. of Antigens (95% CI), %				Responders by Antigen (95% CI), %			
			⩾1 antigen	⩾2 antigens	⩾3 antigens	All 4 antigens	Nef	p17	p24	RT
Day 44	10	46	100 (92.3–100)	100 (92.3–100)	100 (92.3–100)	80.4 (66.1–90.6)	89.1 (76.4–96.4)	91.3 (79.2–97.6)	100 (92.3–100)	100 (92.3–100)
	30	40	100 (91.2–100)	95.0 (83.1–99.4)	85.0 (70.2–94.3)	55.0 (38.5–70.7)	77.5 (61.5–89.2)	65.0 (48.3–79.4)	92.5 (79.6–98.4)	100 (91.2–100)
	90	35	100 (90.0–100)	97.1 (85.1–99.9)	88.6 (73.3–96.8)	65.7 (47.8–80.9)	80.0 (63.1–91.6)	74.3 (56.7–87.5)	97.1 (85.1–99.9)	100 (90.0–100)
Day 60	10	44	100 (92.0–100)	100 (92.0–100)	100 (92.0–100)	90.9 (78.3–97.5)	95.5 (84.5–99.4)	95.5 (84.5–99.4)	100 (92.0–100)	100 (92.0–100)
	30	40	97.5 (86.8–99.9)	92.5 (79.6–98.4)	77.5 (61.5–89.2)	50.0 (33.8–66.2)	77.5 (61.5–89.2)	57.5 (40.9–73.0)	85.0 (70.2–94.3)	97.5 (86.8–99.9)
	90	33	100 (89.4–100)	100 (89.4–100)	87.9 (71.8–96.6)	66.7 (48.2–82.0)	78.8 (61.1–91.0)	75.8 (57.7–88.9)	100 (89.4–100)	100 (89.4–100)
Day 180	10	46	100 (92.3–100)	100 (92.3–100)	84.8 (71.1–93.7)	56.5 (41.1–71.1)	69.6 (54.2–82.3)	73.9 (58.9–85.7)	97.8 (88.5–99.9)	100 (92.3–100)
	30	40	97.5 (86.8–99.9)	92.5 (79.6–98.4)	65.0 (48.3–79.4)	25.0 (12.7–41.2)	57.5 (40.9–73.0)	47.5 (31.5–63.9)	77.5 (61.5–89.2)	97.5 (86.8–99.9)
	90	34	94.1 (80.3–99.3)	85.3 (68.9–95.0)	58.8 (40.7–75.4)	26.5 (12.9–44.4)	44.1 (27.2–62.1)	50.0 (32.4–67.6)	76.5 (58.8–89.3)	94.1 (80.3–99.3)
Day 360	10	44	97.7 (88.0–99.9)	97.7 (88.0–99.9)	84.1 (69.9–93.4)	59.1 (43.2–73.7)	65.9 (50.1–79.5)	79.5 (64.7–90.2)	95.5 (84.5–99.4)	97.7 (88.0–99.9)
	30	40	97.5 (86.8–99.9)	87.5 (73.2–95.8)	62.5 (45.8–77.3)	22.5 (10.8–38.5)	50.0 (33.8–66.2)	47.5 (31.5–63.9)	75.0 (58.8–87.3)	97.5 (86.8–99.9)
	90	35	97.1 (85.1–99.9)	85.7 (69.7–95.2)	54.3 (36.6–71.2)	37.1 (21.5–55.1)	42.9 (26.3–60.6)	54.3 (36.6–71.2)	82.9 (66.4–93.4)	94.3 (80.8–99.3)

[a] T cell responses were evaluated by intracellular cytokine staining after stimulation with p17, p24, reverse transcriptase (RT), and Nef peptide pools. Results were expressed as the percentage of the total $CD4^+$ T cells expressing interleukin 2 and ⩾1 other marker (interferon γ, tumor necrosis factor α, or CD40 ligand). A subject was considered a responder if the antigen-specific $CD4^+$ response was ⩾.03%, the cutoff value. CI, confidence interval.

[b] Subjects with available results.

54.0%–89.8% and 20.0%–50.0% of doses in the F4/AS01 and F4/WFI groups, respectively (grade 3 severity after 0–22.4% of doses in the F4/AS01 groups). No grade 3 fever was reported after the first vaccine doses in the F4/AS01 groups. Grade 3 fever was reported after 14.0%–22.4% of second vaccine doses in the F4/AS01 groups. No solicited local or general grade 3 symptoms were reported in the F4/WFI groups. No differences in reactogenicity were observed between the antigen dose levels in the F4/AS01 groups.

During the 30-day postvaccination period, 60.0%–84.0% of subjects in the F4/AS01 groups reported unsolicited symptoms, compared with 50.0%–70.0% in the F4/WFI groups. In the F4/AS01 groups, unsolicited symptoms (mainly chills and injection site reactions) were considered causally related to vaccination in 30.0%–44.0% of subjects and were of grade 3 severity in ⩽10.0%. All related symptoms were transient and resolved without sequelae, generally within 2–3 days. Six serious adverse events were reported in the F4/AS01 groups, all considered unrelated to vaccination. No subjects died during the study period, and no subject withdrew because of adverse events.

$CD4^+$ T Cell Responses against Homologous Antigens

In all nonadjuvanted groups, the frequency of antigen-specific $CD4^+$ T cells expressing ⩾2 immune markers including IL-2 was below or close to the assay cutoff (data not shown). Very high responder rates were observed in all F4/AS01 groups 2 weeks after the second vaccine dose (day 44) (Table 1). The percentage of responders was highest in the 10-μg F4/AS01 group, with all subjects responding to ⩾3 antigens and 80.4% to all 4 antigens at this time. Responses in the 10-μg F4/AS01 group were broad and directed against all vaccine antigens, with highest response rates to the RT antigen. Vaccine-induced $CD4^+$ T cell responses were long-lived, with 97.7% of subjects in the 10-μg F4/AS01 group still responding to 2 antigens, 84.1% to 3 antigens, and 59.1% to 4 antigens at month 12 (day 360). The magnitude of response for the F4 fusion protein was significantly greater in the 10-μg F4/AS01 group ($P < .0001$ at day 44 vs both 30 and 90 μg). The response to the F4 fusion protein induced in the 10-μg F4/AS01 group was still observable at month 12 (Figure 3). In this group, the median frequency of F4-specific $CD4^+$ T cells producing IL-2 and ⩾1 other marker peaked at almost 1.2% on day 44 and was maintained at .5% at month 12.

Vaccine-induced $CD4^+$ T cells exhibited a polyfunctional phenotype (Figure 4a). The majority of F4-specific $CD40L^+$ $CD4^+$ T cells produced IL-2 alone or in combination with TNF-α and/or IFN-γ. Approximately 50% of F4-specific $CD40L^+$ $CD4^+$ T cells secreted ⩾2 cytokines, and this cytokine coexpression profile was maintained until month 12 (Figure 4b). A similar profile was observed for all individual antigens (data not shown).

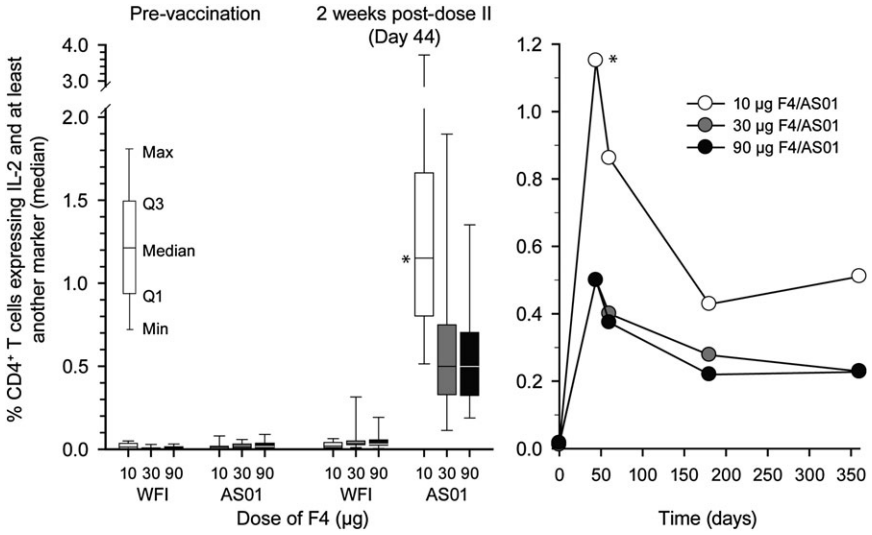

Figure 3. Percentage of $CD4^+$ T cells expressing interleukin (IL) 2 and ⩾1 other marker in response to the F4 fusion protein. F4-specific $CD4^+$ T cell response was estimated from the sum of the specific $CD4^+$ T cell frequencies in response to each individual antigen. Results were expressed as the percentage of the total $CD4^+$ T cells expressing IL-2 and ⩾1 other marker (interferon γ, tumor necrosis factor α, or CD40 ligand). $*P < .0001$ at day 44 for 10 µg versus both 30 and 90 µg groups; statistical comparisons between groups were not performed at any other time. WFI, water for injection.

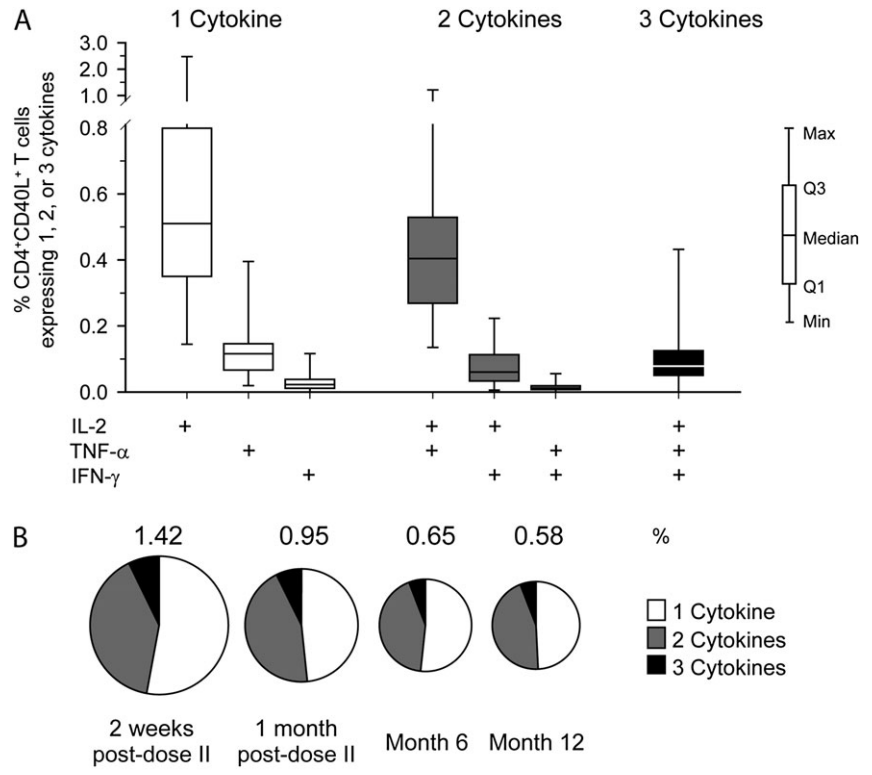

Figure 4. *A,* Cytokine coexpression profile of F4-specific $CD4^+CD40L^+$ T cells in the 10-µg F4/AS01 group at 2 weeks after the second dose (day 44). *B,* Pie charts for all time points in the 10-µg F4/AS01 group. F4-specific $CD4^+$ T cell response was estimated from the sum of the specific $CD4^+$ T cell frequencies in response to each individual antigen. Results were expressed as the percentage of the total $CD4^+CD40L^+$ T cells expressing 1, 2, or 3 cytokines (interleukin [IL] 2, tumor necrosis factor [TNF] α, or interferon [IFN] γ). Pie charts represent percentages as proportions. Values at day 0 were below or close to the assay cutoff (data not shown).

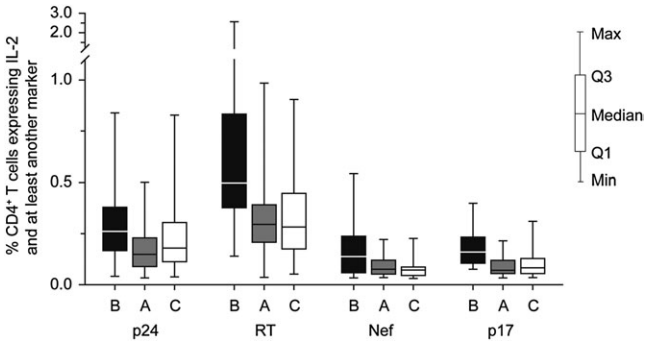

Figure 5. Cross-clade reactivity of $CD4^+$ T cell responses at 2 weeks after the second dose (day 44) in the 10-μg F4/AS01 group among responders. Peripheral blood mononuclear cells collected at days 0 and 44 from subjects in the 10-μg F4/AS01 group were analyzed by intracellular cytokine staining for the expression of CD40 ligand (CD40L) and production of interleukin (IL) 2, interferon (IFN) γ, and tumor necrosis factor (TNF) α, using peptide pools from clades B, A (p17 and p24 from Tanzania, reverse transcriptase [RT] and Nef from Kenya), and C (ZM651) human immunodeficiency virus type 1 (HIV-1) strains. Results were expressed as the percentage of the total $CD4^+$ T cells expressing IL-2 and ⩾1 other marker (IFN-γ, TNF-α, or CD40L). Values at day 0 were below or close to the assay cutoff against each antigen for all clades (data not shown).

$CD4^+$ T Cell Responses against Heterologous Antigens

In the 10-μg F4/AS01 group, HIV-specific $CD4^+$ T cell responses at day 0 were below or close to the assay cutoff for all antigens across all clades (data not shown). Broadly cross-reactive $CD4^+$ T cell responses to p17, p24, RT, and Nef peptide pools of clade A and C were seen at day 44 (Figure 5). The magnitude of the HIV-specific $CD4^+$ T cell response against clade A and C peptides was about half that observed with corresponding clade B peptides across all antigens. Alignment analysis revealed 74%–93% identity between F4 antigens from clade B and the other clades (Table 2). All subjects mounted a response to RT and p24 from clades A and C in line with the high response rates to the corresponding homologous clade B antigens.

$CD8^+$ T Cell Responses

Vaccine-induced $CD8^+$ T cells were not detected by intracellular cytokine staining (data not shown).

Humoral Immune Responses

High immunoglobulin G antibody concentrations against the F4 fusion protein were seen in the AS01 groups (Figure 6). All subjects seroconverted to F4 in the adjuvanted groups, with similar IgG concentrations for all dose levels that persisted until month 12. IgG antibodies were elicited against all individual antigens (data not shown). Very low humoral immune responses were induced in the nonadjuvanted groups.

DISCUSSION

Although the most desirable goal of an HIV vaccine remains the induction of a protective immune response that prevents infection and disease, a vaccine that elicits polyfunctional $CD4^+$ T cell responses may have disease-modifying potential. Vaccine-induced polyfunctional $CD4^+$ T cell responses may contribute to the maintenance of a functional antiviral immune response, preventing progressive $CD4^+$ T cell count decline, reducing or eliminating viral reservoirs, inducing long-term memory, and/or

Table 2. Cross-Reactivity of $CD4^+$ T Cell Responses[a]

Antigen	HIV-1 Clade	Subjects, No.[b]	Responders (95% CI),[c] %	$CD4^+$ T Cells Expressing IL-2 and ⩾1 Other Marker[d]	Amino Acid Identity with Vaccine Antigen, %
p24	B	48	100 (92.6–100)	.26	100
	A	48	100 (92.6–100)	.15	87.1
	C	48	100 (92.6–100)	.18	92.7
RT	B	48	100 (92.6–100)	.50	100
	A	48	100 (92.6–100)	.30	89.8
	C	48	100 (92.6–100)	.28	92.5
Nef	B	46	95.8 (85.7–99.5)	.14	100
	A	23	47.9 (33.3–62.8)	.08	77.7
	C	21	43.8 (29.5–58.8)	.07	81.1
p17	B	41	85.4 (72.2–93.9)	.16	100
	A	28	58.3 (43.2–72.4)	.07	78.8
	C	37	77.1 (62.7–88.0)	.08	74.2

[a] Data include the percentage of responders at 2 weeks after the second dose (day 44) in the 10-μg F4/AS01 group. Peripheral blood mononuclear cells collected at days 0 and 44 were analyzed by intracellular cytokine staining for the expression of CD40 ligand (CD40L) and the production of interleukin (IL) 2, interferon (IFN) γ, and tumor necrosis factor (TNF) α, using peptide pools from clades B, A (p17 and p24 from Tanzania, reverse transcriptase and Nef from Kenya), and C (ZM651) human immunodeficiency virus type 1 (HIV-1) strains. Results were expressed as the percentage of the total CD4+ T cells expressing IL-2 and at least one other marker (IFN-γ, TNF-a, or CD40L). A subject was considered a responder if the antigen-specific $CD4^+$ response was ⩾.03%, the cutoff value.

[b] Subjects with available results.

[c] CI, confidence interval.

[d] Among responders, the median percentage of $CD4^+$ T cells expressing IL-2 and ⩾1 other marker (IFN-γ, TNF-α, or CD40L).

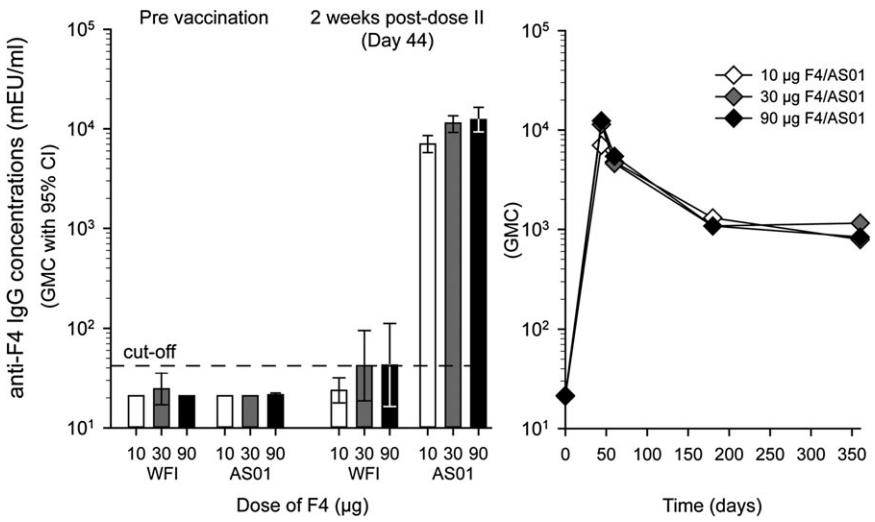

Figure 6. Humoral immune response against the F4 fusion protein. Anti-F4 immunoglobulin G (IgG) antibody concentrations were measured by enzyme-linked immunosorbent assay and expressed as geometric mean concentrations (GMCs in mEU/mL). The assay cutoff for F4 was ⩾42 mEU/mL. WFI, water for injection

conferring LTNP status on infected individuals. We recently assessed the safety and immunogenicity of a gp120/NefTat HIV-1 vaccine candidate formulated with 1 of 3 different Adjuvant Systems in healthy HIV-negative volunteers [20]. Strong, persistent, and broadly reactive $CD4^+$ T cell responses were observed in all vaccine groups but were most pronounced with AS01.

This study assessed the safety and immunogenicity of a novel candidate HIV-1 vaccine consisting of the fusion protein F4 adjuvanted with AS01 in healthy HIV-seronegative volunteers. The F4/AS01 vaccine had an acceptable safety profile, consistent with clinical experience with AS01 in combination with the gp120/NefTat HIV-1 vaccine candidate [20], recombinant hepatitis B surface antigen [25], *Plasmodium falciparum* RTS,S [33], and *Mycobacterium tuberculosis* antigens [34]. The F4/AS01 vaccine elicited a high frequency of F4-specific polyfunctional $CD4^+$ T cells that persisted until month 12. The overall rate of responders was high in all adjuvanted vaccine groups, with responses elicited against all vaccine antigens. However, the most potent $CD4^+$ T cell responses were observed in the lowest antigen dose group (10 µg F4/AS01). Although all subjects who received the F4/AS01 vaccine candidate seroconverted to the F4 antigen, no significant differences in antibody responses were seen between the 3 dose groups. This might indicate important differences in the induction of T versus B cell responses by the candidate vaccine. A possible explanation for this phenomenon would be an influence of the ratio of adjuvant to antigen dose, a hypothesis meriting further exploration in future studies.

Vaccine-induced $CD4^+$ T cells expressed CD40L and produced IL-2 alone or in combination with TNF-α and/or IFN-γ. This is an important and promising observation, because antiviral $CD4^+$ T cells producing multiple cytokines are considered functionally superior to those producing single cytokines [35], and their association with LTNP in HIV-1 infection is well established [15, 16]. Virus-specific polyfunctional $CD4^+$ T cells have also been shown to be important for protective immunity in vaccinated monkeys after simian immunodeficiency virus challenge [36].

HIV-1–specific $CD8^+$ T cell responses were not detected with this vaccine. However, the vigorous $CD4^+$ T cell responses induced may be able to provide the necessary help to $CD8^+$ T cells induced by other vaccine strategies, such as live vectors when combined in a prime-boost regimen [37].

An important consideration in HIV-1 vaccine development is the diversity of the HIV-1 virus worldwide, necessitating the induction of a broadly cross-reactive immune response [38]. The F4/AS01 vaccine, comprising clade B antigens only, was able to elicit broadly cross-reactive $CD4^+$ T cell responses to all 4 antigens derived from clades A and C.

Although these results are encouraging, a potential drawback of a $CD4^+$ T cell inducing vaccine is the fact that HIV-1 preferentially infects activated $CD4^+$ T cells, in particular HIV-1–specific $CD4^+$ T cells [39]. Because any HIV-1 vaccine candidate will eventually depend on some degree of $CD4^+$ T cell induction for an effective antiviral immune response, it may be important to evoke high levels of $CD4^+$ T cells to tip the balance in favor of the immune system. It is reassuring that most vaccines are routinely administered to HIV-infected patients without any safety concern or clinically significant negative effect on disease progression or viral load, despite their activation or induction of $CD4^+$ T cells [40]. A large phase IIb study of a trivalent

recombinant adenovirus-based HIV-1 vaccine did not indicate any correlation between the level of activated $CD4^+$ T cells and enhanced susceptibility to HIV-1 infection or an increased virus load in individuals who became infected during the study [41].

In conclusion, results of this study show the F4/AS01-adjuvanted HIV-1 vaccine to be immunogenic with an acceptable safety profile. Strong polyfunctional, broadly reactive and persistent $CD4^+$ T cell responses were induced with 2 vaccine doses containing 10 μg of F4 protein adjuvanted with AS01. The properties of the immune response suggest that this vaccine candidate merits further evaluation both in a prophylactic setting (alone, in conjunction with an envelope-based antigen, or in combination with other vaccine approaches in a heterologous prime-boost regimen) and as a potentially disease-modifying therapeutic vaccine in HIV-1–infected subjects; a clinical study in such subjects has been initiated (NCT00814762).

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Potential conflicts of interest. G.L.R. was principal investigator for clinical studies of a variety of candidate vaccines for Baxter, GlaxoSmithKline Biologicals, Novartis, and SanofiPasteur and has also performed consulting services for GlaxoSmithKline Biologicals and Novartis. P.B., M.K., M.J., I.C., A.C., M.A.D., G.V., and L.M. were all employees of GSK Biologicals at the time of the study. Ghent University and University Hospital received sponsoring for the conduct of these studies. E.V.B. and F.C. report no conflicts.

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